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A new genus of river snails, *Dalipaludina* (Gastropoda, Viviparidae), endemic to the Yunnan Plateau of SW China

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Abstract

A new genus of viviparid snail, *Dalipaludina* **gen. nov.**, from the Yunnan Plateau of China is described within an integrative taxonomic framework based on data from the mitochondrial COI marker and morphology. *Dalipaludina* can be distinguished from all other viviparid genera by a unique combination shell, operculum and radula characters. Four species are assigned here to the new genus, *Dalipaludina delavayana* **comb. nov.**, *Dalipaludina oxytropoides* **comb. nov.**, *Dalipaludina occidentalis* **comb. nov.**, and *Dalipaludina pyramidella* **comb. nov.**, and one species is newly assigned to *Margarya*, *Margarya dianchiensis* **comb. nov.** The four species of *Dalipaludina* are allopatrically distributed in shallow water lentic habitats at high altitude regions of the Yunnan Plateau.

Key Words

High altitude, lentic habitat, phylogeny, taxonomy

Introduction

River snails (Viviparidae) are widely distributed freshwater gastropods that are found on all continents except for Antarctica and South America. The oldest fossil record of Viviparidae can be traced back to the mid Jurassic. About 28 extant genera and 125 to 150 extant species of this family worldwide are currently recognised (Van Bocxlaer and Strong 2019). China is the country with the highest biodiversity of Viviparidae, harbouring two subfamilies (Bellamyinae Rohrbach, 1937; Viviparinae J. E. Gray, 1847), 13 genera (*Amuropaludina* Moskvicheva, 1979; *Angulyagra* Rao, 1931; *Anularya* Zhang & Chen, 2015; *Cipangopaludina* Hannibal, 1912; *Filopaludina* Habe, 1964; *Idiopoma* Pilsbry, 1901; *Margarya* G. Nevill, 1877; *Mekongia* Crosse & P. Fischer, 1876 *Rivularia* Heude, 1890; *Sinotaia* F. Haas, 1939; *Tchangmargarya* He, 2013; *Ussuripaludina* Zatravkin & Bogatov, 1987; *Viviparus* Montfort, 1810), and at least 40 species (Liu et al 1995; Zhang et al. 2015; Van Bocxlaer et al. 2017).

The Yunnan Province of China, which covers an area of only 394,000 km^2, harbours nine genera (*Angulyagra*; *Anularya*; *Cipangopaludina*; *Filopaludina*; *Idiopoma*; *Margarya*; *Mekongia*; *Sinotaia*; *Tchangmargarya*) including three endemic ones (*Anularya*; *Margarya*; *Tchangmargarya*) and at least 24 species including 14 endemic ones of Viviparidae (Liu et al 1995; Zhang et al. 1997; Zhang et al. 2015). While it is the region with the highest diversity of Viviparidae at both the genus and species level not only in China but also worldwide, recent studies (Du et al. 2011; Zhang et al. 2015; Zhang 2017) have revealed that the diversity of Viviparidae in Yunnan is still underestimated. Based on an integrative study combining molecular and morphological data, we here revise the taxonomy of several viviparid species from the Yunnan Plateau, and describe a new genus. The phylogeny and ecology of the new genus, as well as conservation aspects, are discussed.

Materials and methods

Material

Type specimens of three Viviparidae species from Yunann kept in the Institute of Zoology, Chinese Academy of Sciences, Beijing (**IZCAS**), the Kunming Natural History Museum of Zoology, Kunming (**KIZ**), and the Museum of Comparative Zoology at Harvard University, Cambridge (**MCZ**), as well as other specimens in Museum für Naturkunde, Berlin (**ZMB**), have been examined. From 2011 to 2022, altogether 19 lakes of Yunnan and the surrounding aquatic habitats, including rivers, creeks, springs and wetlands, have been surveyed (Fig. 1A). Newly collected fresh samples of the species belonging to the new genus were collected by Le-Jia Zhang and Jiao-Wei Ning from 2018 to 2022 in northwest Yunnan (Fig. 1B–D). The newly sampled material is stored in ZMB and the private collection of Le-Jia Zhang (**ZLJ**) Jiao-Wei Ning (**NJW**).

Examination of morphology

Shell height (H) and width (W) of only mature and complete specimens were measured with a caliper to a precision of 0.1 mm (Table 1). The morphometric data of

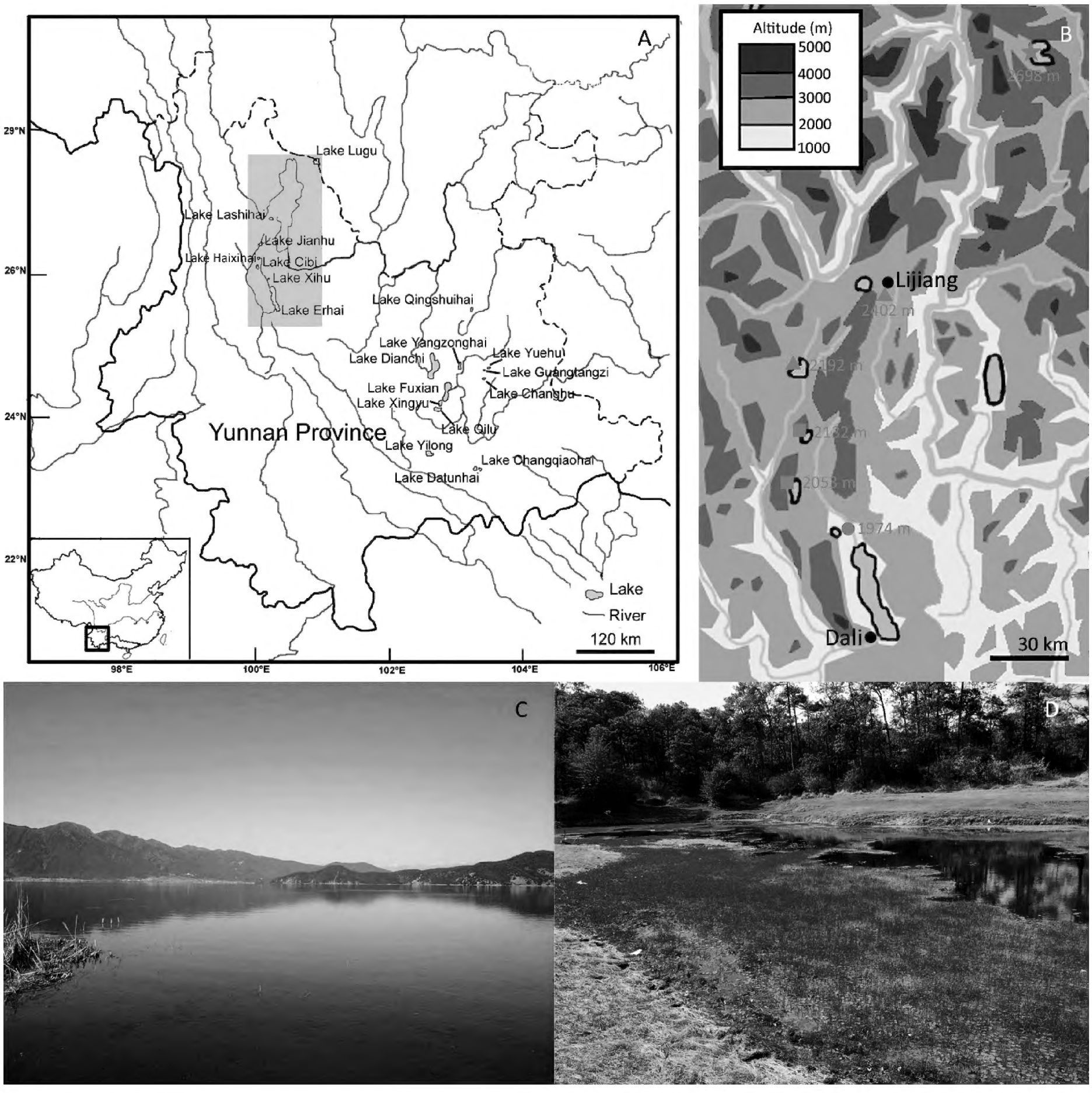

Figure 1. Collecting sites and habitats of *Dalipaludina* gen. nov. in Yunnan, China. **A.** Map of Yunnan Province showing 19 surveyed lakes (adapted from Zhang et al. 2015), study area in northwest Yunnan highlighted in blue, where *Dalipaludina* species were found during surveys; **B.** Map of study area in northwest Yunnan, star–*Dalipaludina oxytropoides*, triangle–*Dalipaludina occidentalis*, square–*Dalipaludina delavayana*, circle–*Dalipaludina pyramidella*; altitude of each collecting site is given; **C.** Environment of one collecting site of *Dalipaludina oxytropoides* in Lake Lugu; **D.** Environment of one collecting site of *Dalipaludina occidentalis* in an unnamed pond in Lijiang.

Dalipaludina oxytropoides kept in ZMB are from Wiese et al. (2022). The specimens were photographed in a consistent orientation using a Sony alpha 7R 4 camera. Radulae and embryonic shells were extracted through dissection; radulae were cleaned by boiling in 1% NaOH solution for half an hour and rinsed with distilled water. Radulae and embryonic shells were coated with gold before scanning electron microscopy with a Zeiss *EVO LS10* scanning electronic microscope. The terms used for describing the operculum are according to Zhang and von Rintelen (2021).

Table 1. Shell measurements of *Dalipaludina* species. Values are arithmetic means (mm).

Species	H	W	W/H
Dalipaludina delavayana (n=25)	58.30 ± 8.30	38.30 ± 5.27	0.70 ± 0.04
Dalipaludina oxytropoides (n=91)	44.60 ± 5.78	32.30 ± 3.82	0.70 ±0.04
Dalipaludina occidentalis (n=27)	50.17 ± 4.17	41.00 ± 2.38	0.82 ±0.05
Dalipaludina pyramidella (n=30)	47.86 ± 7.06	41.34 ± 5.48	0.87 ± 0.05

DNA extraction and amplification

DNA was extracted from the foot tissue of eight individuals (10–20 mg of each individual), using a mollusc-specific CTAB/chloroform extraction protocol (Winnepenninckx et al. 1993). A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified through polymerase chain reaction (PCR) with the following primer pair: LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and COX-B7R, 5′-ACCACCAGCTGGATCAAAAA-3′. (Schultheiß et al. 2011) PCR amplifications were conducted in 25 µl volumes under the following cycling conditions: initial denaturing step at 94 °C for 10 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension step of 10 min at 72 °C. The purification and sequencing were conducted by Macrogen Europe, Amsterdam, Netherlands.

Phylogenetic analysis

Eight new DNA sequences (from eight individuals of two *Dalipaludina* species) have been uploaded to GenBank (accession numbers and museum voucher numbers, see Suppl. material 1). Additionally, three sequences of *Dalipaludina oxytropoides* published by Wiese et al. (2022) and sequences of 17 different genera of the subfamily Bellamyinae (Suppl. material 1) published by Stelbrink et al. (2020) and Hirano et al. (2019a) were downloaded from GenBank; *Viviparus viviparus* of the subfamily Viviparinae was selected as outgroup based on Stelbrink et al. (2020).

Sequences were aligned using MUSCLE as implemented in Geneious Prime 2020 (https://www.geneious.com). Genetic distances were calculated using MEGA X (Kumar et al. 2018). The dataset was tested in MEGA X for the best-fit model of sequence evolution by means of the Akaike and Bayesian information criteria; GTR+G+I was suggested as the best-fitting nucleotide substitution model. This model was employed in a maximum likelihood (ML) analysis conducted by RAxML as implemented in Geneious Prime 2020, with support estimated by 1,000 bootstrap replicates, and a Bayesian inference (BI) analysis performed with MrBayes 3.2.6 (Ronquist et al. 2012) as implemented in Geneious Prime 2020 with four independent chains for 5,000,000 generations, samplefreq = 1,000, burnin = 25%, and confirmed that convergence was reached based on the trace plots generated in Geneious Prime 2020.

Results

Genetic differentiation and phylogeny

COI p-distances between species of *Dalipaludina* are 1.81% to 3.98%; the p-distances between *Dalipaludina* and its closest relative, viz. the *Cipangopaludina*/*Margarya* complex, are 9.22%–14.98%, while p-distances within the *Cipangopaludina*/*Margarya* complex are 0–9.31%.

The phylogenetic trees reconstructed by BI and ML based on COI are highly congruent, therefore only the BI tree is shown (Fig. 2). The new genus *Dalipaludina* is monophyletic and distinct from all other included genera of Viviparidae. The three sequenced species of *Dalipaludina* are each recovered as monophyletic as well. *Dalipaludina occidentalis* is the sister species of *Dalipaludina delavayana*, and *Dalipaludina oxytropoides* is sister to both. *Dalipaludina* is the sister clade of a clade formed by the *Cipangopaludina*/*Margarya* complex, *Celetaia*, *Ussuripaludina*, *Hetergen*, *Torotaia*, *Anularya* and *Sinotaia*.

Systematics

Family Viviparidae J.E. Gray, 1847
Subfamily Bellamyinae Rohrbach, 1937

***Dalipaludina* Zhang, gen. nov.**
https://zoobank.org/6817CC4A-D98A-4D33-B890-49C91F64A1E5

Type species. *Paludina delavayana* Heude, 1889.

Etymology. "Dali" refers to the ancient Dali Kingdom (大理国) mostly situated in modern Yunnan, China; "paludina" refers the assignment to Viviparidae. The recommended Chinese name is "理田螺".

Diagnosis. Shell large, thin but solid; apex acute; teleoconch whorls with strong keel at suture, above suture relatively smooth or with several weak spiral threads or strong spiral cords, many thin and weak spiral threads on base; umbilicus narrow, sometimes bordered by a keel; exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region of operculum smooth, sometimes with small grains; outer marginal tooth with 9 to 11 small sharp cusps.

Comparative remarks. *Dalipaludina* gen. nov. resembles *Cipangopaludina* (widely distributed in East Asia), *Ussuripaludina* Zatravkin & Bogatov, 1987 (endemic to Far East), *Heterogen* Annandale, 1921 (endemic to Japan

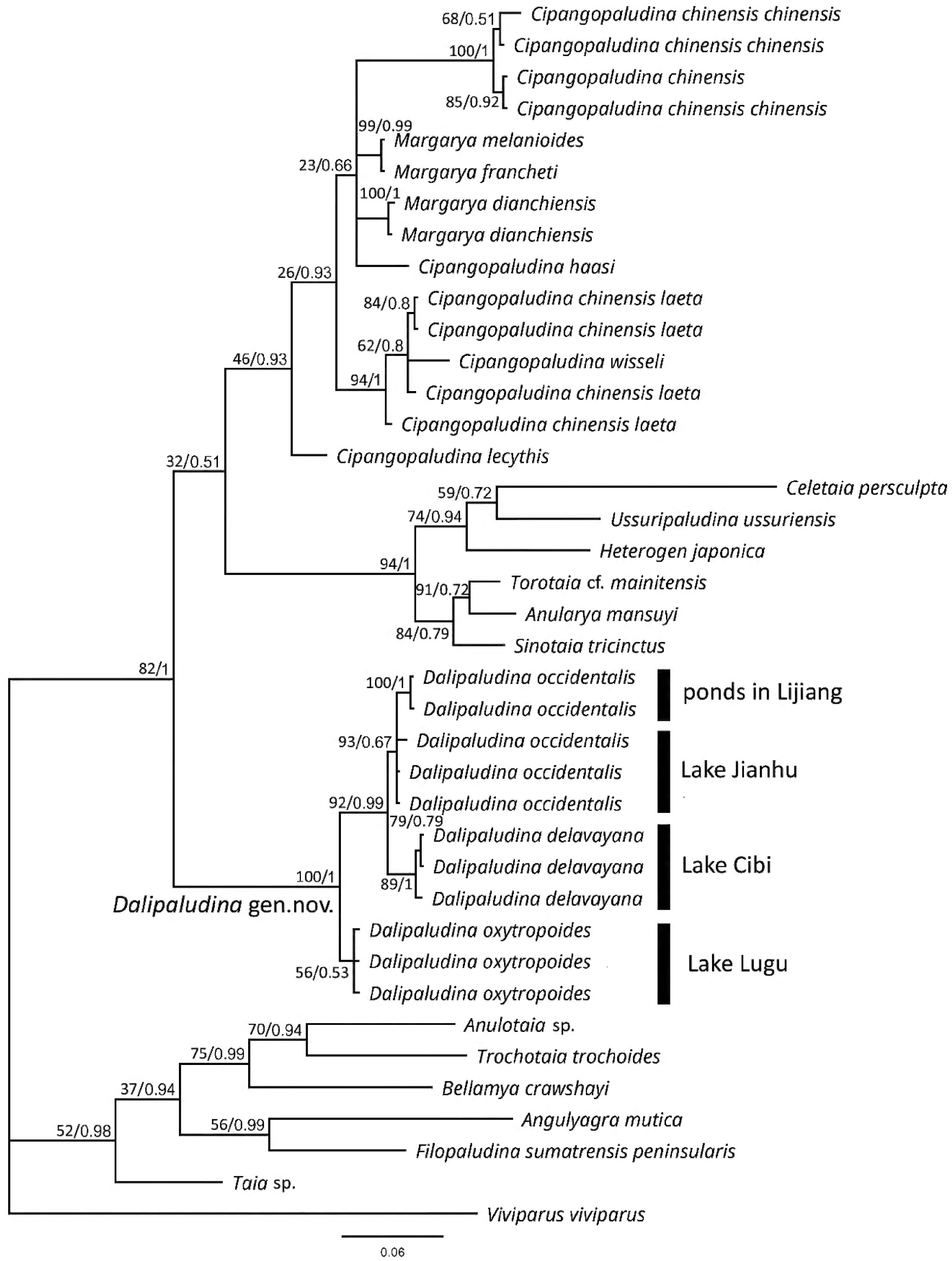

Figure 2. The BI tree based on cytochrome c oxidase subunit I (COI) showing the phylogenetic position of *Dalipaludina* gen. nov. within Viviparidae. Numbers above branches are ML bootstrap values/BI posterior probabilities.

and Korea), and *Torotaia* Haas, 1939 (endemic to Philippines, Sulawesi and New Guinea). It differs from *Cipangopaludina*, *Ussuripaludina*, *Heterogen* and *Torotaia* by having one strong keel at the suture, a shell base with many thin and weak spiral threads, a narrow umbilicus sometimes bordered by a keel, and operculum characters. The molecular phylogeny supports that *Dalipaludina* is distinct from *Cipangopaludina*, *Ussuripaludina*, *Heterogen* and *Torotaia*. The location of the testis in male *Dalipaludina* specimens in the mantle cavity supports the classification of this genus in the subfamily Bellamyinae. There are four known species of *Dalipaludina*.

Distribution. This genus is endemic to the lakes or ponds in northwest to northeast Yunnan, China.

Dalipaludina delavayana (Heude, 1889), comb. nov.

Paludina delavayana Heude, 1889: 47 ("lacu Ta-li fou", Lake Erhai).
Sinotaia delavayana – Qian et al. 2014.

Material examined. 2 ***syntypes***, IZCAS-FG-492553, IZCAS-FG-492554; 1 ***paratype***, MCZ-Mala-167342; 8 specimens in ZMB, collected by Le-Jia Zhang from around 0.5 to 1 metres depth in the north shore of Lake Cibi in Augest 2018, 2 specimens in ZMB, collected by Jiao-Wei Ning from around 1 to 2 metres depth in Lake Haixihai; 17 specimens in NJW, collected by Jiao-Wei Ning from around 1 to 2 metres depth in Lake Cibi.

Description. Shell (Fig. 3A–H) large, conical, thin, greenish yellow to dark olive in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute, spire high; each teleoconch whorls with one strong keel at suture, above suture smooth or with many weak spiral threads, sometimes with additional two to three stronger cords, base of shell with many thin weak spiral threads, weak spiral cords and threads with short periostracal hairs; aperture ovate, less than half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 3I) corneous, ovate, rather thin, yellow, with reddish brown nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (Fig. 7A, B) central tooth with one broad central denticle and with four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth with one broad central denticle and three small sharp cusps on either side; outer marginal tooth with 10 to 11 small sharp cusps.

Remarks. This species differs from the other *Dalipaludina* species by having a larger shell with a higher spiral. It may be difficult to distinguish from the smooth form of *Dalipaludina oxytropoides* from Lake Lugu based on morphology. However, this species is not recorded from Lake Lugu, and can be distinguished from *D. oxytropoides* based on differences in COI gene sequences. The recommended Chinese name of this species is "德拉维理田螺".

Habitat and distribution. shallow water area with sandy and muddy bottoms in Erhai, Cibi and Haixihai, Yunnan, China.

Dalipaludina oxytropoides (Heude, 1889), comb. nov.

Paludina oxytropoides Heude, 1889: 176 ('lacu prope Tchao-tong' = lake near Zhaotong City, Yunnan Province, China).
Vivipara oxytropoides – Kobelt 1909: 128.
Margarya sp. – Du et al. 2012: 45.
Margarya oxytropoides – Zhang et al. 2015: 777; Wiese et al. 2022.

Material examined. 2 ***syntypes***, IZCAS-FG-492586, IZCAS-FG-492587; 1 ***paratype***, MCZ-Mala-167343; 91 specimens in ZMB, collected by Frank Riedel from around 1 to 6 metres depth in all over the Lake Lugu in September and October 2014; 5 specimens in ZMB, collected by Le-Jia Zhang from 1 metre depth in the east shore of Lake Lugu in April 2022.

Description. Shell (Fig. 4A–H) large, conical, thin, greenish yellow to dark brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute; each teleoconch whorls with one strong keel at suture, above suture with many rather weak spiral threads, sometimes with additional two to three strong cords, base of shell with many thin weak spiral threads, weak spiral cords and threads with short periostracum setae; aperture ovate, less than half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 4I) corneous, ovate, thin to relatively thick, yellow to reddish brown, with darker coloured nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth or with small grains.

Radular (Fig. 7C, D) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and three to four small sharp cusps on either side; inner marginal tooth with one broad central denticle and four small sharp cusps on either side; each outer marginal tooth with 10 to 11 small sharp cusps.

Remarks. This species has the most variable shell within *Dalipaludina*. The form with strong cords of this species can be easily distinguished from the other *Dalipaludina* species. It used to be considered as a species of *Margarya* with a distribution in Lake Dianchi as well (Zhang et al. 2015). However, the COI-based phylogeny showed that "*Margarya oxytropides*" from Dianchi is not a species of *Dalipaludina*, but indeed a member of the *Cipangopaludina*/*Margarya* complex. The syntypes of *Paludina oxytropoides* Heude, 1889 from Zhaotong in Northeast Yunnan show a strong keel at the suture, two strong keels above the suture, a relatively smooth shell base with many weak spiral keels, and a narrow umbilicus with a keel around it. It is distinct from the "*Margarya oxytropides*" from Lake Dianchi but quite similar to the strongly keeled form of *D. oxytropoides* from Lake Lugu. Meanwhile, the shell morphology of "*Margarya oxytropides*" from Lake Dianchi matches the description and figure of *Cipangopaludina dianchiensis* Zhang, 1990 endemic to Lake Dianchi (Zhang 1990). Therefore, instead of *D. oxytropoides*, "*Margarya oxytropides*" from Lake Dianchi is here recognised as *Margarya dianchiensis* (Zhang, 1990) comb. nov. *Paludina oxytropoides* Heude, 1889 is reclassified as a member of *Dalipaludina*. The recommended Chinese name of this species is "尖龙骨理田螺".

Habitat and distribution. shallow to medium-depth water area with sandy bottoms in Lake Lugu, Yunnan/Sichuan, China; lakes in Zhaotong, Yunnan, China.

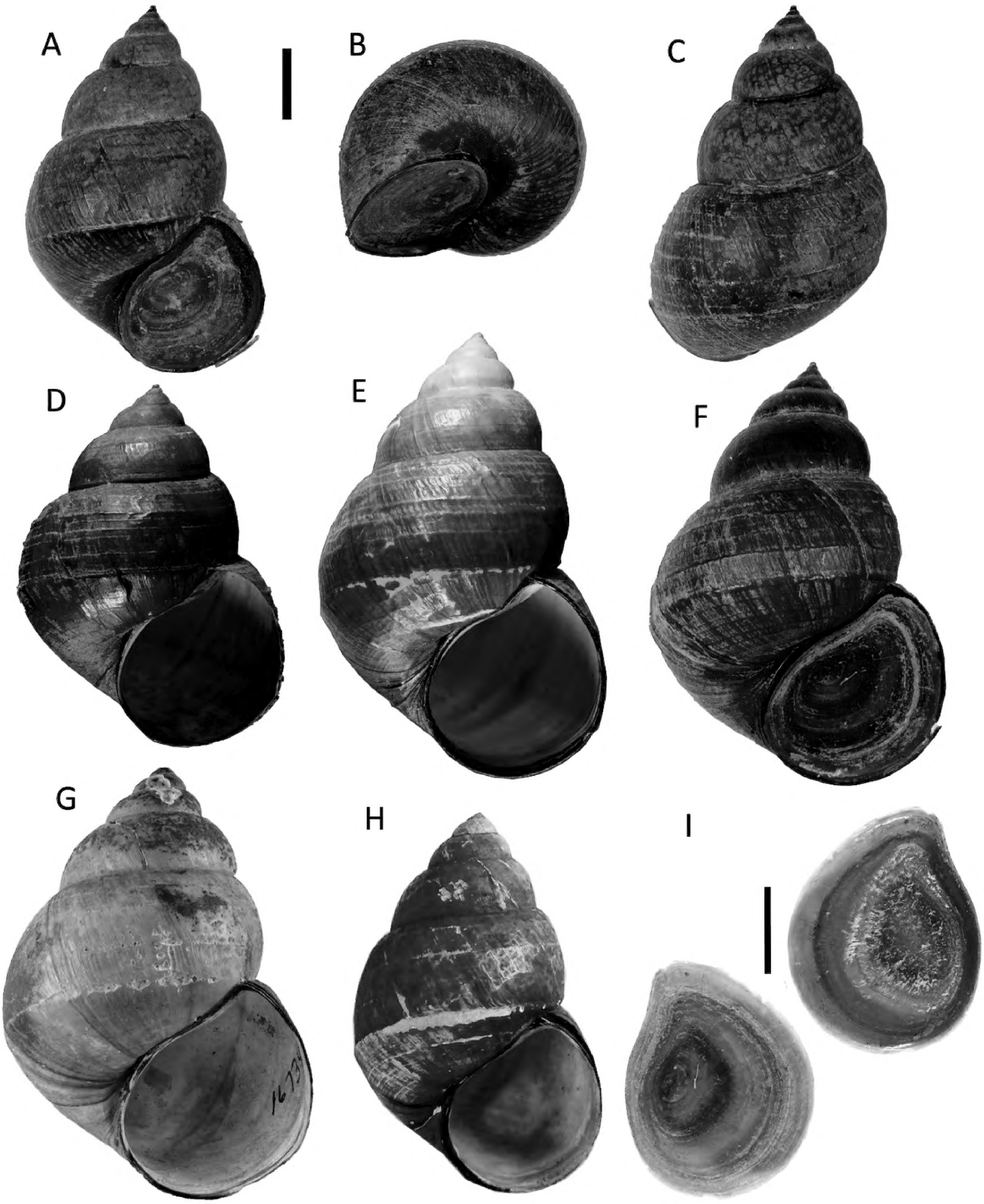

Figure 3. Shell and operculum of *Dalipaludina delavayana*. **A–C.** Specimen collected from Lake Cibi, ZMB Moll. 122707; **D, E.** Varieties collected from Lake Cibi, ZMB Moll. 122707; **F.** specimen collected from Lake Haixihai,LJZ; **G.** Paratype MCZ-Mala-167342, photo taken by Alana Rivera, Museum of Comparative Zoology, Harvard University, President and Fellows of Harvard College; **H.** Syntype IZCAS-FG-492554, photo taken by Kaibaryer Meng, Institute of Zoology, Chinese Academy of Sciences; **I.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–H** with the same scale bar of **A**.

Dalipaludina occidentalis (Annandale, 1924), comb. nov.

Lecythoconcha malleata f. *occidentalis* Annandale, 1924: 415 ("Hoching and Shihku", Heqing County and Shigu town, Lijiang City, Yunnan, China).

Viviparus occidentalis – Yen 1942.

Material examined. 8 specimens in ZMB, collected by local fishermen from 3 to 5 metres depth in the south shore of Lake Jianhu. February 2020; 4 specimens in

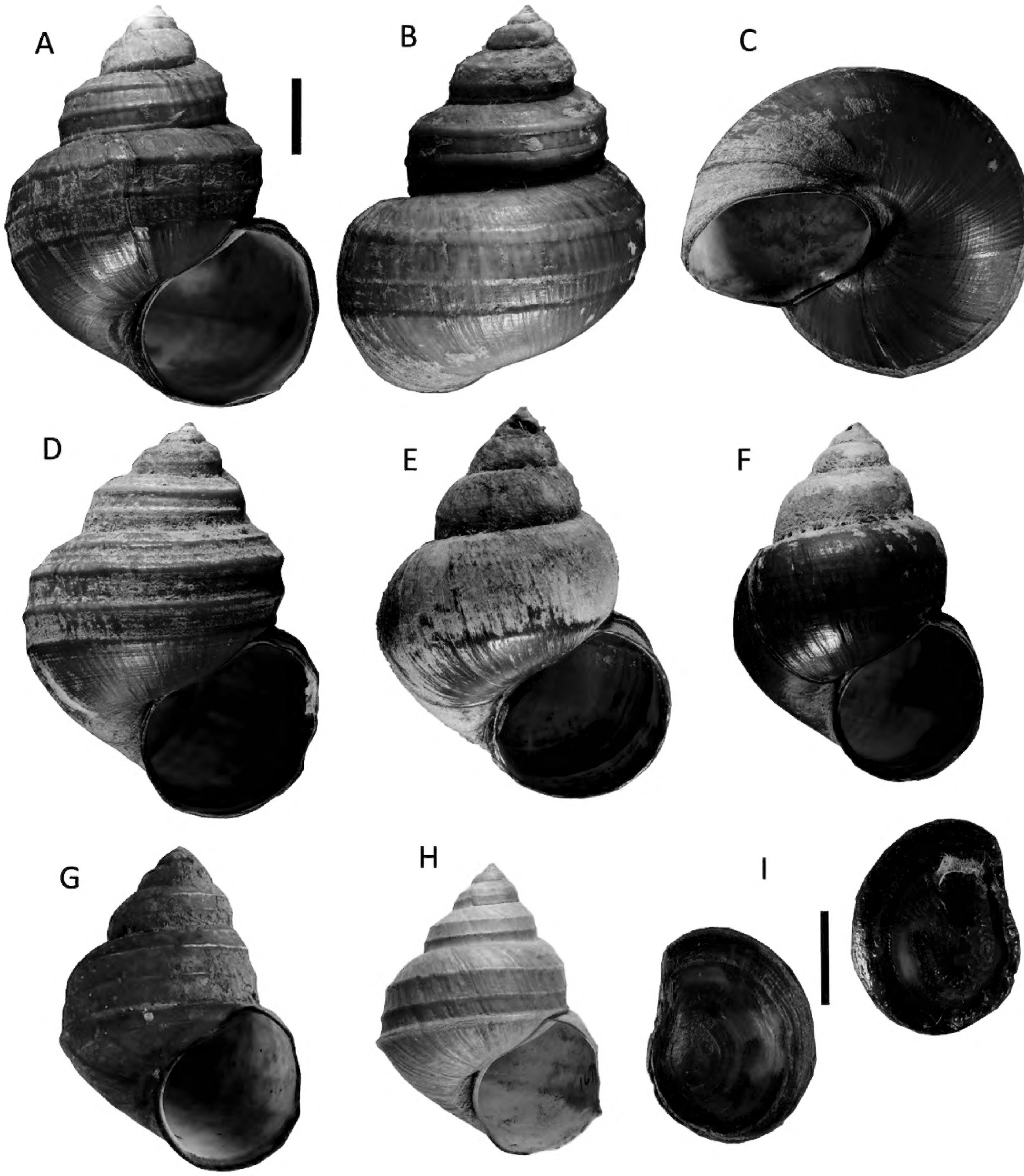

Figure 4. Shell and operculum of *Dalipaludina oxytropoides*. **A–C.** Specimen collected from Lake Lugu, ZMB.Moll. 250844; **D–F.** Varieties collected from Lake Lugu,ZLJ; **G.** Syntype IZCAS-FG-492587 from a lake in Zhaotong, Yunann, photo taken by Kaibaryer Meng, Institute of Zoology, Chinese Academy of Sciences; **H.** Paratype MCZ-Mala-167343 from a lake in Zhaotong, Yunann, photo taken by Alana Rivera, Museum of Comparative Zoology, Harvard University, President and Fellows of Harvard College; **I.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–F, H–I** with the same scale bar of **A**.

ZMB, collected by Le-Jia Zhang from 0.5 metres depth in an unnamed pond on the Sheshan hill in Lijiang City in April 2022; 19 specimens in NJW, collected by Jiao-Wei Ning from Lake Jianhu.

Description. Shell (Fig. 5A–G) large, broadly conical or sub globose, thin, olive to dark brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute; teleoconch whorls inflated, occasionally shouldered, with one strong keel at suture, above suture with many weak spiral threads, sometimes with additional two to three stronger cords, base of shell with many thin weak spiral threads, weak spiral threads and cords with short periostracum setae; aperture ovate, slightly less than half of shell in height,

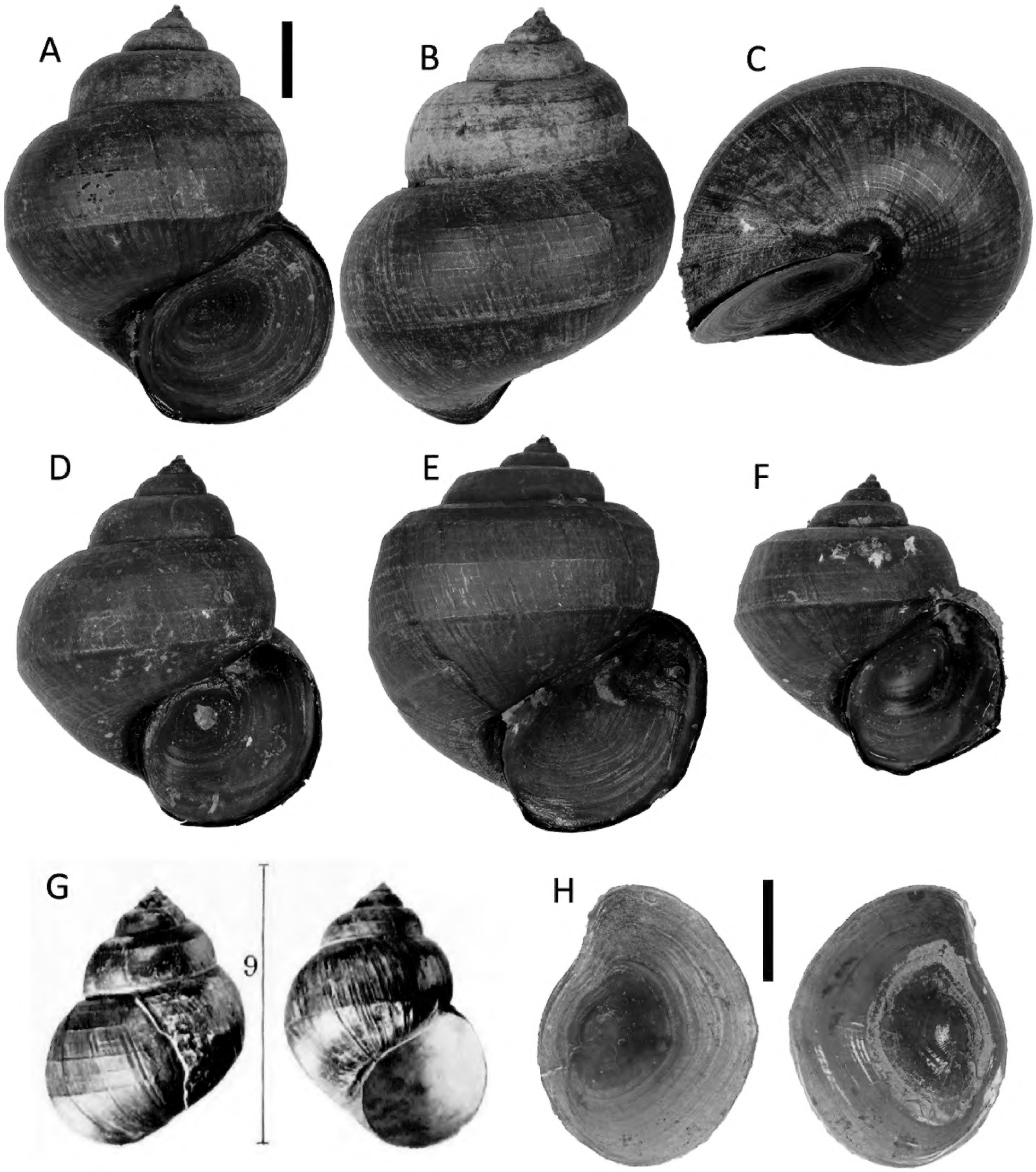

Figure 5. Shell and operculum of *Dalipaludina occidentalis*. **A–C.** Specimen collected from Lake Jianhu, ZMB.Moll. 122712; **D, E.** Varieties collected from Lake Jianhu, ZMB.Moll. 122713; **F.** Sub-adult collected from Lake Jianhu, ZMB.Moll. 122713; **G.** Original figure of the type specimen in Annandale, 1922; **H.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–F** with the same scale bar of **A**.

lip thin and simple, umbilicus narrow, sometimes bordered by akeel.

Operculum (Fig. 5H) corneous, ovate, rather thin, yellow, with red nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (Fig. 7E, F) central tooth with one broad central denticle and four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth with one broad central denticle and four small sharp cusps on either side; outer marginal tooth with 9 to 10 small sharp cusps.

Remarks. This species differs from the other species of *Dalipaludina* by having rather inflated, shouldered whorls. Annandale (1924) presented a photo of the type specimen. The shell morphology and COI phylogeny of the speci-

mens collected from an unnamed pond in Lijiang City, which is very close to the type locality, confirm that this species should be assigned to *Dalipaludina*. The recommended Chinese name of this species is "滇西理田螺".

Habitat and distribution. Shallow ponds with muddy bottoms and abundant aquatic plants in Lijiang City, Yunnan, China; Shallow to medium-depth water area with muddy bottoms in Lake Jianhu, Jianchuan County, Yunnan, China.

Dalipaludina pyramidella (Du, Yang & Chen, 2011), comb. nov.

Trochotaia pyramidella Du, Yang & Chen, 2011: 85–89 (Yousuo village of Er-Yuan County, Yunnan, China).

Material examined. ***Holotype***, KIZ-DLN20100035; 19 ***paratypes***, KIZ-DLN20100036 to KIZ-DLN20100054; 3 specimens in ZMB, 7 specimens in NJW, collected by Jiao-Wei Ning from 1 metre depth in the ponds of Yousuo Village, Eryuan County.

Description. Shell (Fig. 6A–D) large, conical, thin, greenish brown to brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute, spire low; each teleoconch whorls with one strong keel at suture, above suture smooth, with two rings of short periostracum setae, base of shell smooth, with many rather thin weak spiral threads; aperture ovate, almost half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 6E) corneous, ovate, rather thin, yellow or orange, with red nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (according to Du et al. 2011) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either; inner marginal tooth with one broad central denticle and two small sharp cusps on either side; outer marginal tooth with 9 small sharp cusps.

Remarks. This species differs from the other species of *Dalipaludina* by having a relatively smooth shell with lower spire. Du et al. (2011) assigned this species to *Trochotaia* mainly based on the strongly keeled shell with lower spire. However, characters of the operculum, embryonic shells, the reduced number of cusps on the outer marginal teeth (9 in *D. pyramidella* vs 14–16 in *Trochotaia trochoides*) and the distribution in Yunnan strongly support its assignment to *Dalipaludina*. Species of *Trochotaia* have a very different operculum according to Zhang and von Rintelen (2021). This species is also quite similar to *Cipangopaludina miyagii* Kuroda, 1941 endemic to Kaohsiung, S Taiwan, China. It can be distinguished from *C. miyagii* by having a thinner shell, a shell base with many rather thin weak spiral threads, and a narrowly open umbilicus with a keel around it. The recommended Chinese name of this species is "塔形理田螺".

Habitat and distribution. shallow ponds with muddy bottoms and aquatic plant *Ottelia acuminata* (Gagnep.) in Yousuo Village, Eryuan County, Yunnan, China.

Discussion

The molecular and morphological data strongly support that four endemic viviparid species from Yunnan, formerly classified as species of *Cipangopaludina*, *Sinotaia*, *Margarya* or *Trochotaia*, should be assigned to a new genus described here: *Dalipaludina*. The former genus-level classification of these species was primarily based on shell morphology (Du et al. 2011; Qian et al. 2014). However, the shell morphology of freshwater snails can be quite similar due to convergence even between different genera. One remarkable example is the case of three highly sculptured but phylogenetically distinct viviparid genera (*Margarya*, *Tchangmargarya* and *Anularya*) endemic to the plateau lakes of Yunnan (Zhang et al. 2015). Our study has confirmed once again the importance of using an integrative approach in generic classification. In additional to shell characters, the operculum and radula, especially the cusps on the outer marginal teeth, provide important data for genus-level taxonomy of Viviparidae as well. The species-level phylogeny solely based on COI has been considered problematic in Viviparidae (Hirano et al. 2019b). However, at the genus level, it does not seem to be a big problem here (Fig. 2). Our BI tree based on partial COI sequences is broadly consistent with the genus-level topology for the Bellamyinae derived from an analysis of multiple genes in Stelbrink et al. (2020), suggesting that *Dalipaludina* likely falls into clade A of Bellamyinae proposed by Stelbrink et al. (2020).

Four species of *Dalipaludina* can be differentiated based on shell morphology. The scatter plot (Fig. 8) shows that *D. delavayana* has a larger and taller shell; *D. pyramidella* and *D. occidentalis* both have a lower spire, and *D. pyramidella* can be distinguished from *D. occidentalis* based on its less inflated whorls and comparatively smooth shell surface. The COI phylogeny has supported the reciprocal monophyly of the three sequenced species. The populations of *D. occidentalis* from two localities roughly 50 km apart cluster togther in the tree, without obvious geographic structuring (Fig. 2). The keeled, shouldered form of *D. occidentalis* (Fig. 5E) cannot be differentiated from the smooth form based on p-distance or phylogeny of COI as well. Moreover, the four species of *Dalipaludina* have allopatric distributions (Fig. 1B), and we have yet to find any lake or pond with more than one *Dalipaludina* species. Based on these results derived from morphological, molecular, and distribution data, we believe that there are four valid species of *Dalipaludina*.

Intraspecific diversity of shell morphology varies among the four species. *Dalipaludina delavayana* and *D. pyramidella* both display a relatively low intraspecific diversity; morphological diversity within *D. occidentalis* is higher; and that of *D. oxytropoides* is by far the

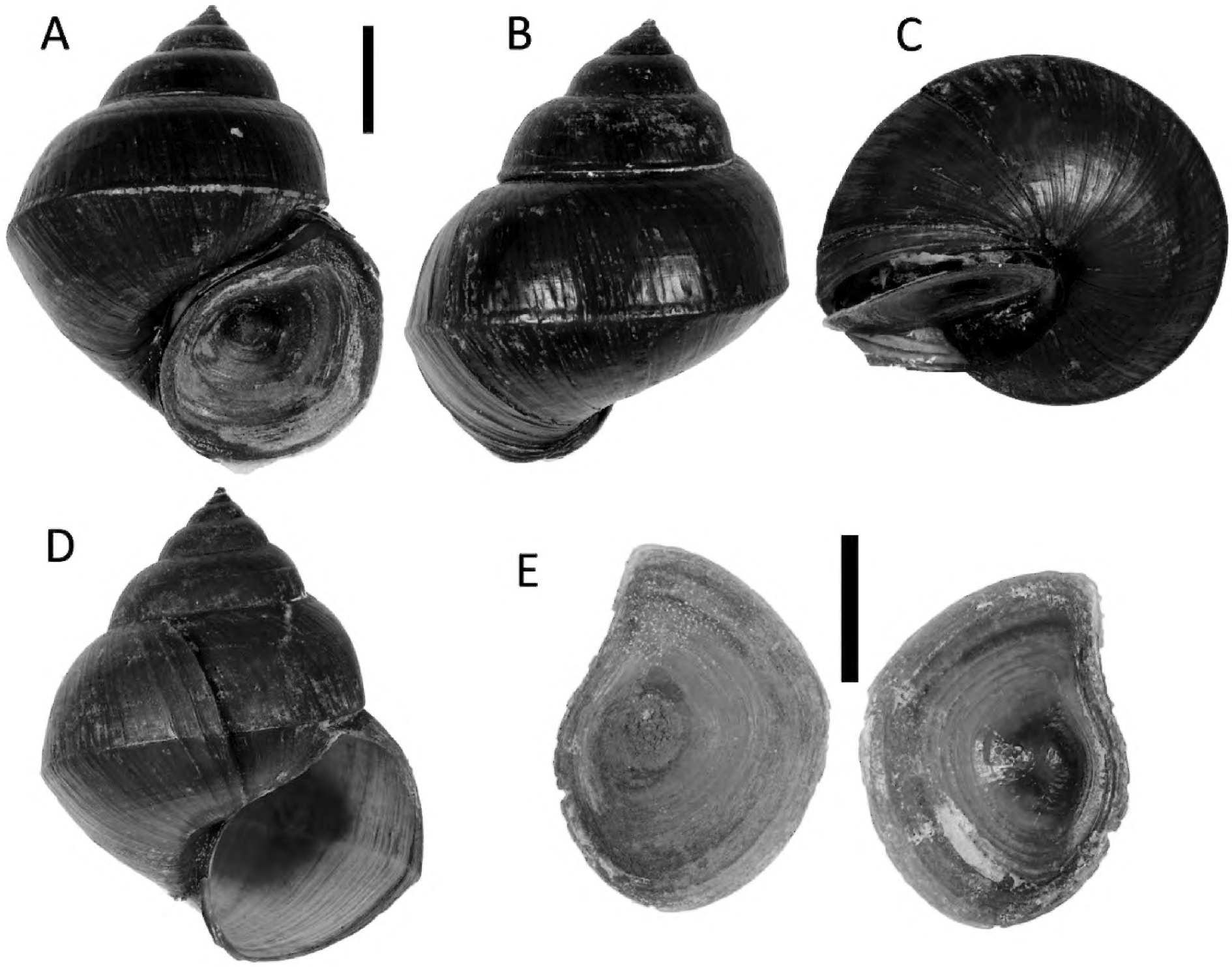

Figure 6. Shell and operculum of *Dalipaludina pyramidella*. **A–C.** Specimen collected from Eryuan County, ZMB.Moll. 122714; **D.** Varieties collected from Eryuan County, ZMB Moll. 122714; **E.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–D** with the same scale bar of **A**.

highest within the genus. An integrative study combining morphometrics and a COI phylogeny of *D. oxytropoides* from Lake Lugu ("*Margarya oxytropoides*" in Wiese et al. 2022) showed that it should be considered a single, but highly variable species. We have also observed many intermediate forms in this species.

In contrast to the other three endemic genera of Viviparidae from Yunnan (*Margarya*, *Tchangmargarya* and *Anularya*) mostly found in medium-depth to deep water (Zhang et al. 2015), *Dalipaludina* displays a preference for shallow water. In addition to large lakes like Lake Lugu or Jianhu, *Dalipaludina* also can be found in small shallow ponds, such as the artificial ponds for farming the aquatic vegetable *Ottelia acuminata* in Eryuan County (*D. pyramidella*) and an unnamed small pond on the top of Sheshan Hill in Lijiang (*D. occidentalis*). *D. oxytropoides* and *D. delavayana* are also more common in shallow water areas than in deeper water. However, *Dalipaludina* is only found in lentic environments. It has never been found in rivers or any other lotic environment.

The lowest altitude where we have found *Dalipaludina* species is in Eryuan County, around 1974 m a.s.l., and one historical record of *D. oxytropoides* in Zhaotong County from ~1920 m a.s.l. (Heude 1886); Lake Lugu harbouring *D. oxytropoides* is ~2698 m a.s.l., which is the highest altitude record of Viviparidae (Fig. 1B). Among the other three viviparid genera endemic to Yunnan Plateau, only *Margarya* is found within a similar but smaller altitudinal range (1886–2192 m a.s.l), while *Tchangmargarya* (1770–1907 m a.s.l.) and *Anularya* (1284–1721 m a.s.l.) inhabit a relatively lower altitude with no overlap with that of *Dalipaludina*. Viviparids from elsewhere in the world occur at much lower altitudes. In conclusion, *Dalipaludina* displays an adaptation to the shallow lentic environment in high altitude regions, and occurs at the highest altitude of any viviparid.

With the discovery of the new genus *Dalipaludina*, the Yunnan Plateau now harbours four endemic genera of Viviparidae, confirming again that it is the most important diversity hotspot of Viviparidae in the world. However, almost all of these species are threatened by human activities such as overharvesting, pollution, and habitat destruction (Yang et al. 2004; Zhang et al. 2015). *D. pyramidella* nearly disappeared from its only known site, the ponds in Eryuan County, during our recent surveys in 2021; the present population of *D. oxytropoides*

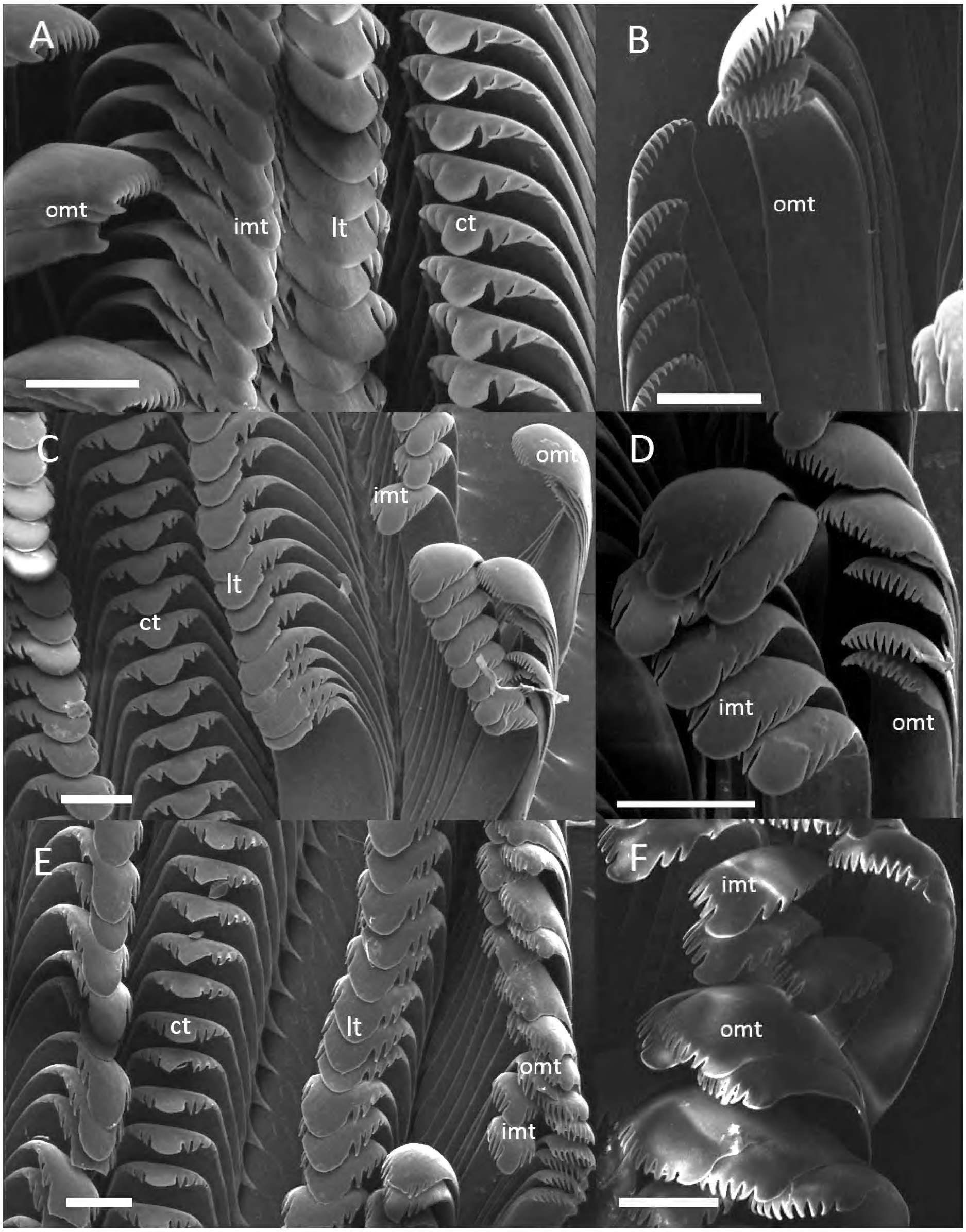

Figure 7. SEM photo of the radula and the protoconch of *Dalipaludina* species. **A, B.** Radula of *Dalipaludina delavayana*, A-ZMB. Moll. 181719, B-ZMB.Moll. 122707; **C, D.** Radula of *Dalipaludina oxytropoides*, ZMB.Moll. 250844; **E, F.** Radula of *Dalipaludina occidentalis*, ZMB.Moll. 122713. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth. Scale bars: 100 µm.

in Lake Lugu has declined dramatically compared to that in the 1990s according to local fishermen. Most endemic viviparid species of *Margarya*, *Tchangmargarya* and *Anulary*a are on the national Red List of China (Wang and Xie 2005), and one species *Margarya melanioides* Nevill, 1877 is a Class 2 species under state protection in the

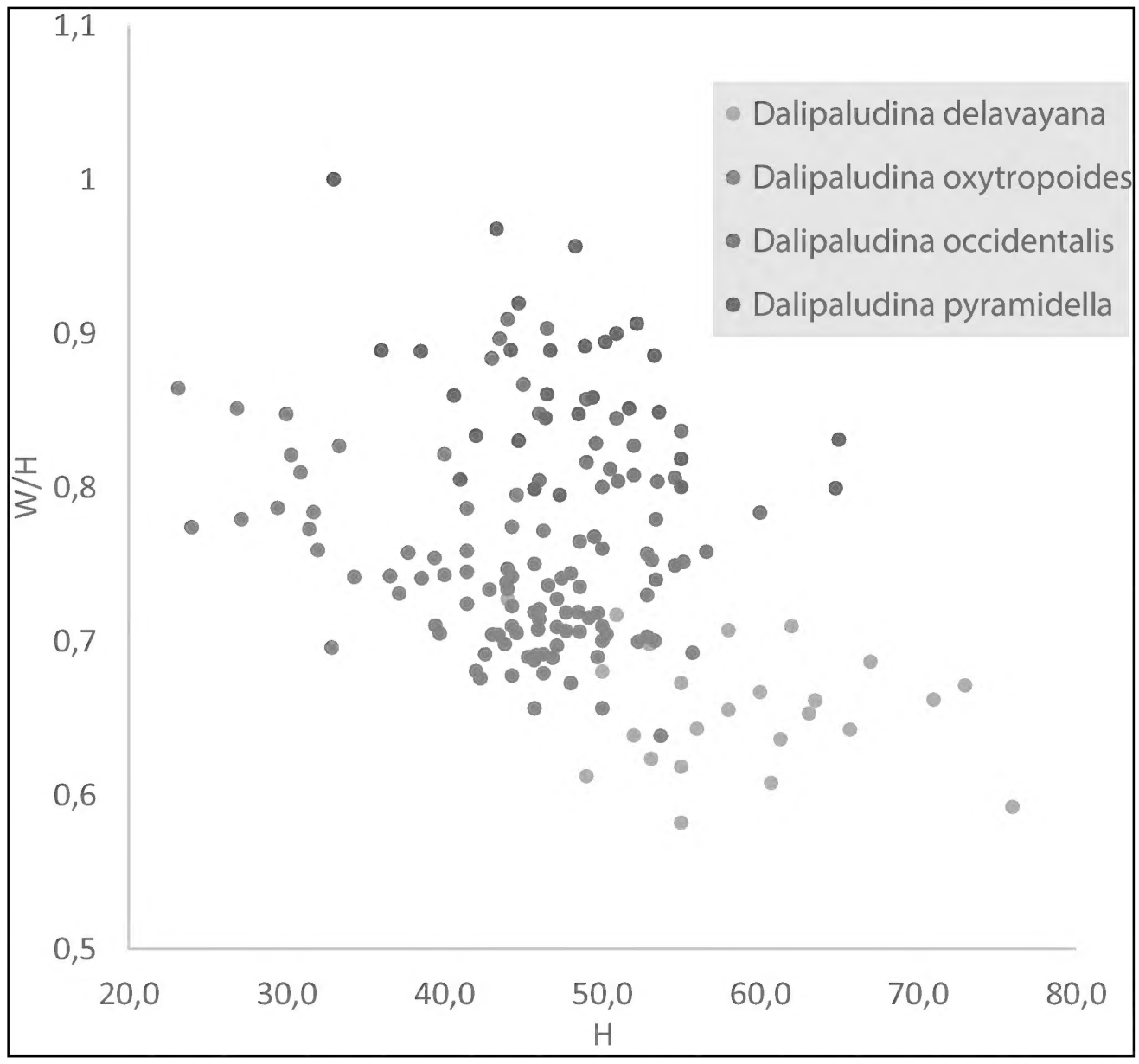

Figure 8. Comparison of *Dalipaludina* species with respect to parameters H (in mm) and W/H.

latest List of State Key Protected Wild Animals of China in 2021. Since all four *Dalipaludina* species are restricted to just one or two lakes or several small ponds, specific conservation measures should be applied urgently for each species based on more detailed population biology and ecology studies. The phylogeny based on genomic data, such as data from exon capture method, can also improve our understanding of the evolution of these rare species endemic to the plateau lentic environment.

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Supplementary material 1

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